

CLAIMS:

- 5 1. A method for constructing a DNA library *in vivo*, comprising:
providing a plurality of host cells;
providing a vector having a first region and a second region;
providing a nucleic acid insert molecule having a first common region
which is homologous with said first region of the vector, a second common region
which is homologous with said second region of the vector, and a library element
encoding region disposed between said first common region and said second common
10 region, wherein when the library element encoding region encodes a naturally occurring
sequence, the first and second regions are not naturally found adjacent to the library
element encoding region;
introducing a vector molecule into each of the host cells;
introducing a nucleic acid insert molecule into each of said cells, wherein
15 a different library element encoding region is introduced into each of said cells; and
allowing homologous recombination and gap repair between a vector
molecule and a nucleic acid insert molecule to occur,
thereby constructing a DNA library.
- 20 2. A method of preparing a plurality of nucleic acid insert molecules,
comprising:
providing a plurality of nucleic acid molecules wherein each of the
nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library
element encoding region, and a second common sequence;
25 providing a plurality of first primers, each of said first primers having a
first region homologous with the first common sequence of the nucleic acid molecule
and having a second region which is not homologous with said first (and preferably
second) common sequence; and
providing a plurality of second primers, each of said second primers
30 having a first region homologous with the second common sequence of the nucleic acid
molecule and having a second region which is not homologous with said second (and
preferably first) common sequence;
forming a reaction mixture which includes said plurality of nucleic acid
molecules, said plurality of said first primers, and said plurality of said second primers,
35 under conditions which provide a plurality of nucleic acid insert molecules having the
following structure, in order from 5' to 3', a second region of said first primer/said first

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common region/a library element encoding region/said second common region/a second region of said second primer,

thereby preparing a plurality of nucleic acid insert molecules.

- 5 3 A method of constructing a DNA library, comprising:
 providing a plurality of nucleic acid molecules wherein each of said
nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library
element encoding region, and a second common sequence;
 providing a plurality of first primers, each of said first primers having a
10 first region homologous with the first common sequence of the nucleic acid molecule
and having a second region which is not homologous with said first (and preferably
second) common sequence;
 providing a plurality of second primers, each of said second primers
having a first region homologous with the second common sequence of the nucleic acid
15 molecule and having a second region which is not homologous with said second (and
preferably first) common sequence;
 forming a reaction mixture which includes said plurality of nucleic acid
molecules, said plurality of said first primers, and said plurality of said second primers,
under conditions which provide a plurality of nucleic acid insert molecules having the
20 following structure, in order from 5' to 3', a second region of said first primer/said first
common region/a library element encoding region/said second common region/a second
region of said second primer;
 providing a plurality of host cells;
 providing a vector having a first region which is homologous with said
25 second region of said first primer, and a second region which is homologous with said
second region of said second primer;
 introducing said vector molecule into each of said host cells; and
 introducing one or more of said nucleic acid insert molecules into each of
said cells,
30 thereby providing a DNA library.

4. The method of claim 3, further comprising allowing homologous
recombination and gap repair between said vector molecule and said nucleic acid insert
molecule to occur.

5. The method of claim 3, wherein said first and second common sequences
are the same.

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6. The method of claim 3, wherein said first and second common sequences are different.

5 7. The method of claim 3, wherein said host cell is a yeast cell.

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8. The method of claim 3, wherein said host cell is a bacterial cell.

9. The method of claim 3, wherein said vector is linearized prior to being
10 introduced into said host cell.

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10. The method of claim 9, wherein said vector is linearized by cleaving between said first and second regions of said vector.

11. The method of claim 3, wherein said second region of said nucleic acid insert molecule is produced by PCR, using primers having a first region which is homologous to the 3' end of the element encoding region and a second region which is homologous to the second region of the vector.

12. The method of claim 3, wherein said first region of said nucleic acid insert molecule is produced by PCR, using primers having a first region which is homologous to the 5' end of the element encoding region and a second region which is homologous to the first region of the vector.

13. The method of claim 3, wherein said second region of said nucleic acid insert molecule is produced by the ligation of adapters having a sequence homologous to the second region of the vector.

14. The method of claim 3, wherein said first region of said nucleic acid insert molecule is produced by the ligation of adapters having a sequence homologous to the first region of the vector.

15. The method of claim 3, wherein said first and second regions of said nucleic acid insert molecule are at least 30 base pairs in length.

16. The method of claim 3, wherein said first and second regions of said nucleic acid insert molecule are at least 40 base pairs in length.

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17. The method of claim 3, wherein said first and second regions of said nucleic acid insert molecule are at least 50 base pairs in length.

5 18. The method of claim 3, wherein said library element encoding region is obtained from a cDNA library other than the one being constructed.

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10 19. The method of claim 18, wherein said library element encoding region is obtained from a cDNA library which is plasmid based .

20. The method of claim 18, wherein said library element encoding region is obtained from a cDNA library which is phage based.

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15 21. The method of claim 3, wherein said library element encoding region is obtained from an mRNA molecule.

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22. The method of claim 21, wherein said mRNA molecule is derived from a cancerous tissue.

20 23. The method of claim 3, wherein said DNA library is screened in a two-hybrid system and wherein said vector includes a transcription factor activation domain.

24. The method of claim 23, wherein said method further comprises,
introducing into said host cell a nucleic acid molecule encoding a hybrid
25 protein, wherein the hybrid protein comprises a transcription factor DNA-binding domain attached to a test protein;
introducing into said host cell a detectable gene, wherein said detectable gene comprises a regulator site recognized by said DNA-binding domain and wherein said detectable gene expresses a detectable protein when said test protein interacts with a
30 protein encoded by the DNA library;
plating said host cell onto selective media; and
selecting for said host cell containing a DNA encoded protein which interacts with test protein.

35 25. The method of claim 3, wherein said DNA library is used for screening and cloning of novel genes.

26. A method of constructing a DNA library for screening in a two-hybrid system, comprising:

providing a plurality of nucleic acid molecules, wherein each of the nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library element
5 encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region homologous with said first common sequence of said nucleic acid molecule and having a second region which is not homologous with said first (and preferably second) common sequence;

10 providing a plurality of second primers, each of said second primers having a first region homologous with said second common sequence of said nucleic acid molecule and having a second region which is not homologous with said second (and preferably first) common sequence;

forming a reaction mixture which includes the plurality of nucleic acid
15 molecules, the plurality of said first primers, and the plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of the first primer/the first common region/a library element encoding region/the second common region/a second region of the second primer;

20 providing a plurality of host cells;

providing a vector having a first region which is homologous with the second region of the first primer, and a second region which is homologous with the second region of the second primer, wherein said vector further includes a transcription factor activation domain;

25 introducing a vector molecule into each of said host cells;

introducing one or more of the nucleic acid insert molecules into each of said cells under conditions which allow for recombination and gap repair to occur;

introducing into said host cell a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein includes a transcription factor DNA-binding domain attached
30 to a test protein;

introducing into said host cell a detectable gene, wherein said detectable gene comprises a regulator site recognized by the DNA-binding domain and wherein said detectable gene expresses a detectable protein when the test protein interacts with a protein encoded by the DNA library;

35 plating said host cell onto selective media; and

selecting for said host cell containing a DNA encoded protein which interacts with test protein.

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27. A kit allowing the interchangeable use of a DNA library in more than one application, comprising:

5 a plurality of first PCR oligonucleotide primers, each of said first PCR primers having a first region homologous with the first common sequence used in the construction of said DNA library, and a second region homologous with a first region of a vector required for a particular application;

10 a plurality of second PCR oligonucleotide primers, each of said second PCR primers having a first region homologous with the second common sequence used in the construction of said DNA library, and a second region homologous with a second region of a vector required for a particular application; and instructions for use.

15 28. An oligonucleotide primer having a first region homologous with a linker sequence used in the construction of a DNA library, and a second region homologous with an insertion region of a vector required for a particular application.

29. A method for screening a subject for the existence of a lesion in a gene encoding a particular protein, comprising:

20 obtaining a tissue sample from said subject;
preparing from said tissue, a plurality of nucleic acid insert molecules having a first region, a library element encoding region and a second region, wherein said library element encoding region encodes said protein or portion thereof;

25 providing a vector having a first region which is homologous to the first region of said nucleic acid insert molecule and a second region which is homologous to the second region of said nucleic acid insert molecule, wherein said vector is suitable for use in an assay which detects the interaction between two proteins;

providing a host cell suitable for use in an assay which detects the interaction between two proteins;

30 introducing into said host cell said nucleic acid insert molecule, and said vector;

performing said assay which detects the interaction between two proteins, thereby screening subjects for the existence of a lesion in a gene encoding a particular protein.

35 30. The method of claim 29, wherein the plurality of said nucleic acid insert molecules are prepared by PCR using a first and a second primer, said first primer

The first region comprises a sequence of claim 29, where

31. The method of claim 29, wherein said assay is a two-hybrid assay.

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